Contact has been established between the Australian National Research Council and the following:

(i) The Executive of the Council for Scientific and Industrial Research.
(ii) The Adjutant General.
(iii) The Director General and Secretary of the Ministry of Munitions.
(iv) The Director General and Secretary of the Department of Supply and Development.
(v) The Chief Draughtsman, Maribyrnong.
(vi) The Controller of Materials, Ministry of Munitions.
(vii) The Assistant Controller of Industrial Chemicals.
(viii) The Chairman, Medical Equipment Control Committee.
(ix) The Department of Information.
(x) The N.S.W. Contracts Board.
(xi) The Army Medical Services.

The result of these contacts is that several scientific problems arising out of the nation's war effort have been dealt with by scientists in universities and elsewhere. The following are examples of this:

(*a*) A committee of chemists has, in cooperation with the Assistant-Controller of Industrial Chemicals, Ministry of Munitions, prepared a list of chemicals needed in Australia for industrial and analytical work and not manufactured here. The committee has initiated research work on some of these chemicals.

(*b*) The A.N.R.C. has initiated work on the preparation of fire-foam stabilizers (formerly imported) from peanut shells in Australia. This manufacture is about to begin on a scale large enough to supply all Australian requirements and, if required, to export abroad.

(*c*) The A.N.R.C. has initiated research work on the production of sensitizers, essential for engineering drawings, from chemicals available in Australia. In Maribyrnong alone over 2 million square feet *per annum* of this paper are used in munition manufacture, and it is all imported. Research to produce sensitizer has so far been successful, and at present the A.N.R.C., with the cooperation of the Ministry of Munitions, is about to conduct tests which will determine whether an Australian-made sensitizer can replace the imported material in munitions work.

(*d*) The Drug Sub-committee of the Association of Scientific Workers is affiliated to the Australian National Research Council and conducts its negotiations with the Medical Equipment Control Committee largely through the Australian National Research Council's representative. With the help of these negotiations several pieces of work of national importance have been done by the sub-committee.

(*e*) A sub-committee of the A.N.R.C. in collaboration with the Medical Equipment Control Committee has been examining the situation regarding the supplies of essential veterinary drugs.

(*f*) A sub-committee of the A.N.R.C. investigated the possibility of local agar manufacture, with the cooperation of C.S.I.R., and made recommendations concerning the importation of agar supplies for essential pathological and scientific work.

(*g*) A liaison officer has been appointed between Eastern Command and the Australian National Research Council to assist in the solution of scientific problems arising in the Army. The liaison officer, Major Pulling, is attached to the General Staff of Eastern Command.

(*h*) The A.N.R.C., with the agreement of the executive of C.S.I.R. and the vice-chancellors of Australian universities, will examine and comment upon the annual reports of the expenditure of the Commonwealth Research Grant to universities, and will publish summaries of the reports.

(*i*) Through its contact with the N.S.W. Contracts Board, the A.N.R.C. has been asked to give advice on technical difficulties in the fulfilment of war contracts.

(j) The A.N.R.C. has pressed for the appointment of entomologists to the A.I.F.

(*k*) The A.N.R.C. called a conference of representatives from the Australian universities and from C.S.I.R. to discuss the need for training workers in agricultural economics, in view of the need for collecting information before agricultural policy is determined.

# SPECIAL ARTICLES

## EQUILIBRIA IN AN ANTIGEN-ANTIBODY REACTION

ACCORDING to a theory of immune precipitation developed elsewhere,[1] an equilibrium constant for the *initial reactions* between antigen (G) and antibody (A)

$$G + xA = GA_x$$

may be written

$$K_v^* = \frac{[\text{Satisfied valences}]}{[\text{free valences of G}]\,[\text{valences of free A}]}$$

$$= \frac{G_o x}{G_o(g-x)Ca}\ \text{mol}^{-1}\ \text{liters}$$

where "valence" is defined by the identity, $G_o$ is the initial concentration of G, C is the equilibrium concentration of A, $g$ is the maximal valence of G, and $a$ is the maximal valence of A. This formulation requires three assumptions: that the initial reactions are bimolecular, that the strength of the forces binding a

[1] A. D. Hershey, *Jour. Immunol.*, 42: 455, 1941.

* The constant k ($= 1000/K_v$) employed in (1) refers to the dissociation of the A–G complex, and to the unit volume $10^6$ ml.

specified A molecule to G are not modified by the presence of other bound A molecules, and that the forces binding different A molecules are the same. The second assumption differentiates this equilibrium from that between $H^{\cdot}$, $HCO_3^{-}$, and $CO_3^{--}$, and the third distinguishes it from adsorption on heterogeneous surfaces.

It was shown further[1] that precipitation occasions a minimal disturbance of the initial equilibrium when $x = g-x = g/2$, so that, approximately,

$$K_v = \frac{x}{Ca(g-x)} = \frac{1}{aC} \qquad [1]$$

after precipitation at this optimal ratio.

$K_v$ does not refer to concentrations in the ordinary sense, but measures directly the strength of the valence forces, in terms permitting comparisons between different systems. This constant, if validly determined, is therefore of considerable immunologic significance.

It is the object of the present paper, however, to obtain the usual

$$K_c = \frac{[GA_x]}{[GA_{x-1}]\,[A]} \text{ mol}^{-1} \text{ liters} \qquad [2]$$

which has a different utility.

From data of Heidelberger and Kendall cited in reference 1, table 2, we estimated that the reaction of ovalbumin in rabbit antiserum 3.87II could be described by the parameters $a = 2$, $g = 5$, $K_v = 2.9 \times 10^5$. That is, at the point $x = 2.5$, $C = 1.7 \times 10^{-6}$ mols per l. We wish now to characterize, by means of $K_c$, the initial bimolecular reactions possible to this system (Table 1).

TABLE I

EQUILIBRIUM CONSTANTS AND FREE ENERGY DATA FOR AN OVALBUMIN-ANTIOVALBUMIN REACTION (DATA OF HEIDELBERGER AND KENDALL[a])

| Reaction | $K_v$* | $K_c$ | $-\triangle F^\circ = RT \ln K_c$ |
|---|---|---|---|
| | $\times 10^5$ | $\times 10^5$ | cal. |
| $\frac{1}{x}G + A = \frac{1}{x}GAx$ .. | 2.9 | ... | 7000† |
| $G + A = GA$ .... | " | 29 | 8300 |
| $GA + A = GA_2$ ... | " | 12 | 7800 |
| $GA_2 + A = GA_3$ ... | " | 5.8 | 7400 |
| $GA_3 + A = GA_4$ ... | " | 2.9 | 7000 |
| $GA_4 + A = GA_5$ ... | " | 1.2 | 6500 |

Standard state: Aqueous solutions of the order $10^{-5}$ M in serum diluted 1 : 5 with 0.15 M NaCl.
Temperature: 0 C.
[a] M. Heidelberger and F. E. Kendall, *Jour. Exp. Med.*, 62: 697, 1935.
* The constancy of $K_v$ for the successive reactions is in part an assumption (see A. D. Hershey, *Jour. Immunol.*, 1941, on which the validity of the computed $K_c$'s depends.
† $RT \ln K_v$, *i.e.*, the decrease of free energy associated with formation of the G–A bond. The remaining values have the usual significance.

It is evident from the assumptions made above that, for the mean value $x = 2.5$, the mol fractions of the various $GA_x$'s participating in the initial equilibrium may be obtained from the successive terms of the expansion $(p+q)^n$ where $n = 5$, $pn = 2.5$, $p = 0.5$ and $q = 1-p$. Accordingly,

mol fraction G = 0.03125
" " GA = 0.15625
" " $GA_2$ = 0.31250
" " $GA_3$ = 0.31250
" " $GA_4$ = 0.15625
" " $GA_5$ = 0.03125

from which since $[A] = 1.7 \times 10^{-6}$, the values of $K_c$ for the successive reactions may be obtained by [2].

This method is alternative to another, which is less obvious but considerably more convenient when $g$ and $n$ are large. Thus for the single reactions

$$GA_{x-1} + A = GA_x$$

$$K_v = \frac{x[GA_x]}{(g-x+1)\,[GA_{x-1}]\,2[A]} = \frac{x}{2(g-x+1)} K_c \qquad [3]$$

The $K_c$'s computed by [3] are identical with those given by [2].

In Table 1 these values are listed for the successive reactions, together with $\triangle F^\circ = -RT \ln K$ referred to the arbitrary standard state represented by dilute solutions of the reactants in serum diluted with 0.15 M NaCl.

The striking feature of the ovalbumin-antiovalbumin reaction is the very large value of the equilibrium constants, which could scarcely be determined at all except for the large molecular size of antibody. This is, however, in keeping with the well-known "irreversible" character of the A–G reaction.

It should be noted that the equilibrium constants of the successive reactions decrease very markedly as $x$ increases, which accounts for the partial recovery of antibody by dissociation from precipitates formed in the region of A excess[2,3,4] and failure in other regions.[3,4,5] This result, as here interpreted, does not imply "a graduated variability in the firmness of union"[2] of successive A molecules, a conception rather generally accepted at present.[6,7] On the other hand, this possibility can not be excluded by ignoring it, as is done by the assumptions made in the present theory. Experimentally, one observes only a minor variation of $K_v$ computed for different points in the reaction range, and this has been attributed to the secondary effects of precipitation.[1] The significant fact is that the results achieved appear to justify the simpler assumptions. It remains to be seen within what quantitative limits this is true.

[2] F. M. Huntoon, *Jour. Immunol.*, 6: 117, 1921.
[3] M. Heidelberger and E. A. Kabat, *Jour. Exp. Med.*, 67: 181, 1938.
[4] A. D. Hershey, G. Kalmanson and J. Bronfenbrenner. Unpublished.
[5] P. H. DeKruif and J. H. Northrop, *Jour. Gen. Physiol.*, 5: 139, 1922–23.
[6] J. R. Marrack, "The Chemistry of Antigens and Antibodies." Great Britain Medical Research Council, Special Report Series No. 230, 1938.
[7] Szu C. Liu and H. Wu, *Chinese Jour. Physiol.*, 16: 97, 1941.

The question of the reversal by dissociation of the *biologic effects* of immune reactions may be somewhat clarified in view of the variations of $K_c$ for the successive reactions. Thus, other parameters being the same, a system in which the "neutralization" of a molecule of antigen brought about by reaction with considerably less than $g$ molecules of A might appear irreversible, whereas in a system in which neutralization requires many ($\sim g$) molecules of A, reactivation should be readily demonstrable. In practice, success depends on whether the necessary degree of dilution, and the necessary lapse of time, are experimentally feasible.

The free energy data offered are, of course, subject to those errors,[1] of unknown magnitude at present, affecting the measurement of $K_v$. The values appear to be reasonable. In fact, Boyd *et al.*[8] recently assumed that $\triangle F^\circ = -10^4$ cal. for a similar reaction "which goes very nearly to completion but may be reversed experimentally."

We are now studying the phage-antiphage equilibrium,[4] which is in some respects more amenable to measurement, with the expectation of obtaining a more complete thermodynamic description of this typical immune reaction.

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## EFFECT OF INSULIN ON PYRUVIC ACID FORMATION IN DEPANCREATIZED DOGS[1]

PREVIOUS work on man has revealed an increase in pyruvic acid in the blood following the ingestion of glucose.[2] In diabetic subjects no increase in blood pyruvic acid occurred under the same conditions. The administration of insulin together with glucose to these patients resulted in an increase in blood pyruvate.[3] In order to study the relationship between insulin and pyruvic acid formation 27 experiments have been performed on 14 depancreatized dogs. The animals were maintained with insulin and pancreatin until 72 hours before each observation. The method for estimating pyruvic acid was modified to eliminate interference by the 2, 4 dinitro-phenylhydrazone of acetoacetic acid.[4,2]

Experiments on normal animals disclosed a significant rise in blood pyruvate over the fasting value after the intravenous injection of 2 gm of glucose per kg body weight. In depancreatized dogs there was no rise in blood pyruvate after a similar injection of glucose. When insulin was administered simultaneously with the glucose a marked rise in pyruvate occurred (see table I representing a typical experiment) reaching its maximum from one to three hours after the injection. If a second injection of glucose was made four hours after the administration of insulin a second rise in pyruvic acid took place. When the blood sugar level was raised to 750 to 950 mg per cent. for 3 to 5 hours by a continuous infusion of a 5 per cent. glucose solution, (300 ml per hour) after a preliminary injection of 2 gm glucose per kg, an elevation of blood pyruvate occurred despite the absence of insulin. The blood pyruvate under these conditions reached a constant level within one or two hours. The injection of insulin after three hours of glucose infusion produced a further rise in blood pyruvate.

TABLE I

BLOOD PYRUVIC ACID AND BLOOD SUGAR AFTER THE INTRAVENOUS INJECTION OF GLUCOSE (2 GM PER KG) INTO A DEPANCREATIZED DOG (BOTH EXPERIMENTS WERE PERFORMED ON THE SAME ANIMAL)

| Time | No insulin | | 40 units insulin | |
|---|---|---|---|---|
| | Blood pyruvic acid mg per cent. | Blood sugar mg per cent. | Blood pyruvic acid mg per cent | Blood sugar mg per cent. |
| Before injection | 1.28 | 380 | 1.14 | 337 |
| 10 min. after " | 1.23 | 775 | 1.35 | 725 |
| 20 " " " | 1.28 | 662 | ... | ... |
| 30 " " " | 1.29 | 572 | 2.52 | 445 |
| 45 " " " | 1.28 | 515 | ... | ... |
| 60 " " " | 1.19 | 478 | 3.46 | 279 |
| 90 " " " | 1.22 | 438 | ... | ... |
| 120 " " " | ... | ... | 3.12 | 243 |

In agreement with previous observations[5] the rate of removal of intravenously injected pyruvic acid (1 gm per kg as sodium pyruvate) from the blood was the same in normal and depancreatized dogs.

It may be concluded from these experiments that, in the depancreatized dog, insulin increases the formation of pyruvic acid after the administration of glucose.

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[8] W. C. Boyd, J. B. Conn, D. C. Gregg, G. B. Kistiakowsky and R. M. Roberts, *Jour. Biol. Chem.*, 139: 787, 1941

[1] Aided by grants from the John and Mary R. Markle Foundation and the Williams-Waterman Fund of the Research Corporation.

[2] E. Bueding, M. H. Stein and H. Wortis, *Jour. Biol. Chem.*, 140: 697, 1941.

[3] E. Bueding, H. Wortis and H. Fein: Unpublished observations.

[4] D. Klein, *Jour. Biol. Chem.*, 137: 311, 1941.

[5] E. Flock, J. L. Bollman and F. C. Mann, *Jour. Biol. Chem.*, 125: 49, 1938.